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Effect of metabolite of *Tamarix aphylla* against liver fibrosis in Balb-c mice

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Abstract

The main focus of this research was the characterization of ethanol extract of *Tamarix aphylla* leaf (EETAL) to explore the antioxidant profile and hepatoprotective activity *against* CCl_4 liver injury in mice. The EETAL was used for phytochemical study; phenolics and flavonoids content (185.66± 3.05 mg GAE/g and 194.66±1.52 mg QE/g). *In vivo* studies, mice were allocated randomly into four groups, each group comprises of six mice. Liver was injured by CCl_4 of (2-4) groups to analyze the liver marker enzyme. Lowest value of superoxide dismutase (SOD), Glutathione peroxidase and catalase (10.26±1.07, 1.05 ±0.08, 6.91 ± 0.30) in CCl_4 treating group but normal in G_4 group EETAL treated group (SOD, Gpx and catalase: 19.08±1.25, 2.47 ± 0.17, 12.41±0.30) remains nearer to the control group (SOD, Gpx and catalase: 25.37± 1.11, 2.96± 0.37, and 12.56±0.92) or silymarin group (SOD, Gpx and catalase: 21.18± 1.05, 2.56 ±0.15, 12.64±0.54) respectively. The values of lipid peroxidation and MDA (22.51 ± 1.15, 7.49± 0.15) were significantly higher in CCl_4 treating group as compare to the normal group (LPO and MDA: 10.39± 0.05, 2.49± 0.03) and decrease in group 4 treated with EETAL (13.75 ±0.10, 2.34± 0.09). The high level of serum marker enzyme in CCl_4 group (AST, ALT, ALP, and Bilirubin total: [(213.6±5.4, 264.16±5.9, 306.3±4.9, 1.41±0.3) IU/L]. The EETAL recover the serum level [(AST: 43.35±2.65, ALT: 53.5± 2.8, ALP: 178.33±2.1) IU/L]. The histopathological examination showed improvement in cellular architecture with EETAL. The results of present research demonstrated the protective effect of EETAL against liver fibrosis in mice.

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Introduction

Liver diseases are major health problems all over the world. Viral infections: Hepatitis, environmental pollutants, toxic industrial chemicals, alcohol, and aflatoxin are the main contributor to liver diseases (Juan *et al.*, 2019). Liver fibrosis involves a gradual damage to cell, dysfunction of the liver parenchyma cell, scar formation as well as accumulation of ECM protein (Novo *et al.*, 2015). In vitro liver injury induces by some chemical agents such as paracetamol (Liu *et al.*, 2011), acetaminophen (McGill *et al.*, 2012), acetylsalicylic acid (Raza *et al.*, 2011) and CCl_4 respectively. Hence, acute injury is associated with the complete restoration of the liver function and architecture. The chronic injury is reflected by continuously extend and prolonged duration of acute injury and results in end-stage of liver failure or hepatic cancer (Brautbar *et al.*, 2002; Malhi *et al.*, 2008). Therefore, liver injury is also caused by CCl_4 induction; it produces a chemical liver injury. Lu and co-workers studied the mechanism of liver injury in mice (Lu *et al.*, 2016). CCl_4 is converted to trichloromethyl radical CCl_3 via a cytochrome P-450, which form unbalanced CCl_3 and tri-chloromethyl peroxyl CCl_3O_2 radicals (Knockaert *et al.*, 2012; Chiu *et al.*, 2018). These two trichloromethyl and peroxy radicals are able to bind with the protein and lipids and extracting an H atom from anhydrous lipid, and cause lipid peroxidation and resulted in liver destruction (Desai *et al.*, 2012). *Tamarix aphylla* (L.) is utilized as therapeutic plant throughout the world (Lefahal *et al.*, 2010; Emad and Gamal, 2013). *Tamarix aphylla* is the most familiar species in Pakistan. Bark, leaves, and stem are ideally used in the favor of different diseases with no side effect.

Thus leaves of *Tamarix aphylla* were used in the treatment of many infectious ailments (Panhwar and Abro, 2007; Marwat *et al.*, 2008), revealed good antibacterial activities (De Victorica and Galván, 2001). Aqueous extract of *Tamarix aphylla* has been shown an antioxidant activity (Auribie 2011). *Tamarix aphylla* ethanolic extract has significant antipyretic and analgesic activities. It also contains alkaloids, flavonoids, cyanogenic glycosides and tannins (Prakash *et al.*, 2004). The good antioxidant and antifibrotic activity of medicative plants are owing to the existence of metabolites in them (Mohsin *et al.*, 1989). A recent study revealed that many phytochemicals such as phenolic compounds, tannins, and alkaloids are present in *Tamarix aphylla* leaves. In this perspective, the present study was aimed to evaluate the ethanolic extract of *Tamarix aphylla* leaves for the anti-fibrotic activity first time.

Materials and methods

Procurement of material

The *Tamarix aphylla leaves* were collected from Agriculture University Faisalabad, Pakistan. Leaves were thoroughly washed with distilled water to eliminate dust particles, air- dried at room temperature, and grounded into a fine powered (Vijay *et al.*, 2011).

Method of extraction

About 300 g of the powdered was soaked in 1200ml solvent ethanol: H_2O (80:20) at room temperature for 48 h in a beaker and covered with the aluminum foil. Extraction was carried out Sonicated-assisted stirring (DSA-100-SK1-2.8L) at 25C° for 10 mint.

The concentrated extract was obtained using a rotary evaporator (BUCHI ROTAVAPOR R-200) under reduced pressure at 45 C°. Semi-solid extract was kept in sterile sample tubes and stored at -4 °C until testing and analyzed (Hussain *et al.*, 2012). Crude concentrated extract was weighted to estimate the percentage yield by using the formula.

$$\%\text{age yield} = \frac{\text{weight of plant extract}}{\text{weight of powder plant material}}$$

Phytochemical evaluation

This was carried out according to the methods described by (Auribie 2011). Tannins: 250mg ethanol extract was mixed with 10 ml of double-distilled water and filter the solution. Take a filtrate (2ml) and add $FeCl_3$ (2ml). Formation of the blue and black precipitate showed that the presence of Tannin and phenols.

Alkaloids: 10 ml methanol adds 200mg ethanol extract, shakes and filtered. After filtration take 2ml of filtrate + few drops of 1%HCl and mixture was steam then add 5-6 drops of Wagner's reagent or dragendroff's reagent. Brown red precipitate showed that the presence of alkaloids.

Saponins: Take a 200mg of ethanol extract and add 10ml of double distilled water and filtered. After filter the solution 1 ml of filtrate and add 5ml of distilled water. Frothing persistence showed that the presence of saponin. Ternoipeds: Taking a 250 mg of extract, add 10ml of double distilled water and filter. After filtration take 2ml of filtrate and 2ml of acetic anhydride and few drops of conc. H_2SO_4 Spontaneously blue or green ring formed indicates the presence of terpenoids (Farah *et al.*, 2013).

Phenolics: 100mg of extract was mixed with 5ml of Folin-ciocalteu (10%) and then add 3ml of Na_2CO_3 (20%) and then incubate for an hour.

The resulting blue color complex was formed and checks the absorbance at 765nm. The complete content of phenolic acid in ethanol extract of *Tamarix aphylla* leaves was expressed as Gallic acid equivalents (GAE) and calculated by the following formula.

T = C x V / M

Flavonoids: The complete content of flavonoids in ethanol extract of *Tamarix aphylla* leaves were determined by using the method (chang *et al.*, 2006). 100mg of extract was mixed with 2ml of ddH_2O and adds 0.5 ml of 5 % $NaNo_2$ solution and incubated for 5 mints. After incubating the sample then add 0.5 ml of 10% $AlCl_3$ solution. Pink or red color indicates the presence of flavonoids. Check the absorbance at 510nm after incubation for 15minutes.

The whole content of flavonoids of the extracts was expressed as catechin equivalents from the linear regression curve of catechin (Chang *et al.*, 2006).

HPLC analysis

Quantitative analysis of plant extract was accomplished by (HPLC).The sample for HPLC analysis was prepared by a method directed by Hi-Technology laboratory, University of Agriculture, Faisalabad. Take 50mg of *Tamarix aphylla* extract dissolved in 16 ml of DDH_2O and 24ml of HPLC grade methanol, then shake for 5 minutes and after that added 10ml of 6 Molar HCl. Then the sample was heated in oven at 90 C° for two hours and then filtered through micro-filter 0.2-0.4 microns. The sample was run through HPLC (Shimadzu, Japan). Flavonoids and phenolics compound were analyzed by using Shim-pack CLC-ODS (C18), 25cm × 4.6mm, 5µm. Reverse phased chromatography technique was applied. The mobile phase used comprised of two gradients A and B. Gradient A: (H_2O: Amino acid-94:6, pH 2.27) Gradient B: (Acetonitryl 100%), 0-15 mint= 15%B, 15-30%= 45%. 30-45=100%, B at flow rate 1 ml/min. The detector used was UV-Visible detector (SPD-10AV) at 280 nm at room temperature (Sultana *et al.*, 2008).

Efficacy studies

Animal with 6-8 weeks old were taken from the NIH (National Institute of Health) Islamabad. Twenty four experimental mice were divided into 4 groups comprising of six animals per each group. The entire animals were placed in the animal house of pharmacy department of Govt. College University Faisalabad. The animals were delivered standard dietary conditions i.e. room temperature of 25± 1 ºC; humidity 40-50% and provided 12:12 day and night cycle till the sacrifice of all animals (Nagalekshmi *et al.*, 2011).

Acute toxicity study

The acute toxicity study was based on a previous study (Lu *et al.*, 2012). Six Balb-c mice divided into two groups for plant extract of *Tamarix aphylla* and standard silymarin were fasted overnight and the orally administrated of different doses 50,100, 200, 250 and 500mg/g b.wt of silymarin and ethanol extract of *Tamarix aphylla* . Animals were observed for a period of eight days for severity of any toxic sign and mortality (Surendra *et al.*, 2012).

Induction of hepatic injury

Liver injury was induced in mice by administering CCl_4 (Merck, Germany) intraperitoneal in the lower abdomen. The CCl_4 mix with the olive oil with the ratio of 1:4 v/v at the dose of 1ml/g b.wt on every (Monday and Thursday) for a month. Hepatic injury was monitored by increasing the biochemical marker enzymes (SGOT, SGPT, alkaline phosphate and Bilirubin (Nasir *et al.*, 2013).

Experimental design

The Balb-c mice were divided into four groups of six mice each. Group 1 served as the normal control i.e., they received normal saline for the duration of 30 days. Group 2 was served as a hepatotoxic group. Group 3 (hepatoprotective agent control) and received silymarin tablets (200mg/kg) daily for a period of 4 weeks. Group 4 was served as the treatment group received plant extract. Ethanolic extract of *Tamarix aphylla* leaves was mixed with distilled water and administered by intragastric administration at a dose 500mg/kg b.wt for four weeks with a 24 hours interval. Dose was calculated by using the formula $m_1c_1=m_2c_2$. At the end of study the mice were killed. Blood was taken from the cervical area of the neck at fasting condition. Separation of serum from blood by centrifuging the sample at 14000rpm for ten mints at 4°c and after that biochemical analysis was performed. The tissue of liver was instantly taken out, dry and weighed and some part of liver was fixed in formalin solution (10%) for further histopathological study (Kim *et al.*, 201). A 10% of homogenate liver tissue was taken for antioxidants profile.

Biochemical analysis

The obtained serum was used to estimate the following liver function test like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT),serum alkaline phosphatase (SALP), total bilirubin, direct bilirubin, Indirect bilirubin with the standard method. These biochemical tests were estimated using commercial kits according to manufacturer's protocol (Rajib *et al.*, 2009).

Measurement of CCl_4 mediated Oxidative stress

The antioxidant activity such as superoxide dimutase (SOD), catalase, glutathione peroxidase and Malondialdehyde (MDA) and lipid peroxidation (LPO) were assayed in the homogenate hepatic tissue of control and experimental group of mice.

Microscopic evaluation of liver histopathological analysis

Microscopic study was carried out to examine the histopathological changes in the liver cell. For this purpose, the part of the liver tissues was cut into 2-3 pieces approximately $5mm^3$ sizes and preset in 10% formalin solution and undergo dehydrate process in gradient ethanol. 5 µm thick slides of liver tissues were slice and stained with hematoxylin-eosin dye.

All the section of the tissues were examined under microscope for analyzing the altered architecture due to their CCl_4 liver injury and improved architecture with *Tamarix aphylla* extract and standard drug Silymarin (Ramesh *et al.*, 2011).

Data analysis

The Statistics 8.1 was introduced for the statistical data analysis. All treatments were performed in triplicate and all the results are representing in Mean± SD. Significant differences between groups were assessed by the least significant difference (LSD) analysis was used to determine the significant difference at p-value of < 0.05 (Steel *et al.*, 1997).

Results and discussion

Phytochemical study

The results in Table 1 showed that the phytochemical analysis of *Tamarix aphylla* leaves, tannin, alkaloids, terpenoids, saponins, flavonoids, and phenolic compound was present.

Phytochemical analysis showed that the presence of phenolics (185.66± 3.05 mg GAE/g) and flavonoids (194.66±1.52 mg QE/g).These results compare with the previous results aqueous extract of *Tamarix aphylla* was used to identify the phytochemical screening (Auribie, 2011).

Table 1. Phytochemical analysis of ethanol extract of *Tamarix aphylla* leaves.

Test	Reagents	Result
Tannins	1 % $FeCl_3$	+
Alkaloids	Wagners or dragendroff's	+
Saponins	Shaking	+
Phenolics	1 % $FeCl_3$	+
Flavonoids	$NaNo_2$, $AlCl_3$	+
Terpenoids	Acetic anhydride, H_2SO_4	+

Analysis of ethanolic extract of Tamarix aphylla leaves by HPLC

HPLC technique most frequently used for analysis of phenolic acid and flavonoids in plant extract (Khoddami *et al.*, 2013). *Tamarix aphylla* leaves extract was conducted to get HPLC chromatograms as shown in (Fig. 1-2). HPLC chromatogram shows the peaks of Quercetine (6.25±0.01 ppm), Kaemferol (520.81±0.011ppm), Galic acid (75.56±0.02 ppm), caffeic acid (46.14±0.01ppm), syringic acid (19.72±0.01ppm), ferulic acid (72.12±0.015ppm) cinnamic acid (15.85±0.015ppm) and ellagic acid (80.45±0.012 ppm) are shown in (Table 2).

Table 2. HPLC profiling of *Tamarix aphylla* Leaves.

Compound Name	Amount (ppm)	Reten.time	Area %	Area [m.V.s]
Quercetin	6.25±0.01	3.007±0.005	1±0.1	118.233±0.02
Kaemferol	520.81±0.011	1.800±0.002	94.40±0.01	1680.726±0.003
Galic acid	75.56±0.02	4.567±0.001	18.1±0.01	2099.355±0.01
Caffeic acid	46.14±0.01	12.647±0.008	8.6±0.1	1001.542±0.003
Syringic acid	19.72±0.01	16.607±0.003	6.8±0.1	789.792±0.002
Ferulic acid	72.12±0.015	21.7±0.05	8.7±0.1	1009.613±0.001
Cinamic acid	15.85±0.015	25.007±0.001	3.9±0.1	453.527±0.003
Elligic acid	80.45±0.012	5.52±0.001	3.5±0.1	463.452±0.002

Values are expressed as mean ± SEM; for every three replicate.

All these values were compared with the retention time of the standards; the peak in HPLC was identified.

Considering the peak area of the reference compounds, the concentration of each phenolic compound in the extracts was identified and it was reported as ppm of extract. Ferulic acid is a hydroxycinnamic acid and present in many plants. It acts as an antioxidant and neutralizes the free radical (nitric oxide, superoxide and peroxide) which could generate the oxidative damage of cell membrane and DNA (Kamila *et al.*, 2018). Adel Mahfoudhi also observed polyphenols in *Tamarix aphylla* stem. The experimental data were compared with the literature and commercially standard are available. For reliable results the compound is detected in research work were recognized were also previously identified in other species belonging to a genus of *Tamarix*, which include quercetin, gallic acid, ellagic acid and flavones (Mahfoudhi *et al.*, 2014). Most of the reported compound in genus of the *Tamarix* also identified in this research work phenolics and flavonoids which include gallic acid, caffeic acid (Amina *et al.*, 2018). Garcia reported that the ellagic acid prevents liver toxicity induced by CCl_4 or alcohol through mechanism of free radical scavenging activity, chelation of divalent ions and modulation of CYP450 enzymes activity (Garcia and Zazueta 2015). *Tamarix aphylla* leave extract was the first time isolates the compound cinnamic acid and ferulic acid in this study.

Table 3. Effects of ethanol extract of *Tamarix aphylla leaves* on liver marker enzyme.

Treatment	AST (IU/L)	ALT(IU/L)	ALP (IU/L)	BIL.T (IU/L)	BIL.DIR (IU/L)	BIL.IND IU/L)
G1	29.667±1.5^{d}	36.333±3.7^{d}	179.5±5.5^{b}	0.68±0.0^{b}	0.26±0.05^{d}	0.48±0.07^{b}
G2	213.6±5.4^{a}	264.16±5.9^{a}	306.3±4.9^{a}	1.41±0.3^{a}	0.56±0.16a	1.18±0.17a
G3	35.33±3.82^{c}	43.46±3.26^{c}	163.83±5.88^{c}	0.55±0.04^{b}	0.33±0.16c	0.41±0.14^{b}
G4	43.35±2.65^{b}	53.5± 2.8^{b}	178.33±2.1^{b}	0.75±0.03^{b}	0.25±0.05^{b}	0.48±0.09^{b}

It is assumed that the ethanolic effect of *Tamarix aphylla* leaves on liver protection or liver fibrosis is related to free radical scavenging activity. The literature proved that phytotherapeutic agents extracted from *Tamarix aphylla* leaves had strong inhibitory effect against CCl_4 liver injury. According to our research work the leaves of *Tamarix aphylla* showed good antifibrotic activity due to the presence of antioxidants.

Table 4. Effects of ethanol extract of *Tamarix Aphylla* on tissue enzymatic antioxidant activity.

Treatment	Catalase	GPX	SOD	LPO	MDA
G1	12.56±0.92^{a}	2.96± 0.37^{a}	25.37± 1.11^{a}	10.39± 0.05^{c}	2.49± 0.03^{c}
G2	6.91 ± 0.30^{b}	1.05 ±0.08^{d}	10. 26±1.07^{d}	22.51 ± 1.15^{a}	7.49± 0.15^{a}
G3	12.64±0.54^{a}	2.56 ±0.15^{b}	21.18± 1.05^{b}	12.77 ±0.30^{b}	2.40 ±0.05^{c}
G4	12.41±0.30^{a}	2.47 ± 0.17^{b}	19.08 ±1.25^{c}	13.75 ±0.10^{b}	2.34± 0.09^{c}

Effect of extract on mice liver weight and body weight

The leaves extract of *Tamarix aphylla* affect the mice liver weight and body weight are presented in (Fig.3-4).The results show that the highest body weight and liver weight in group1 (37.5±2.4; 6±0.7) but the lowest body weight and liver weight (31.66±1.34; 3.83±0.28) was observed in group 2 (CCl_4 group), which specifies that the liver tissue had been rigorously damaged, induced by exposure to CCl_4.

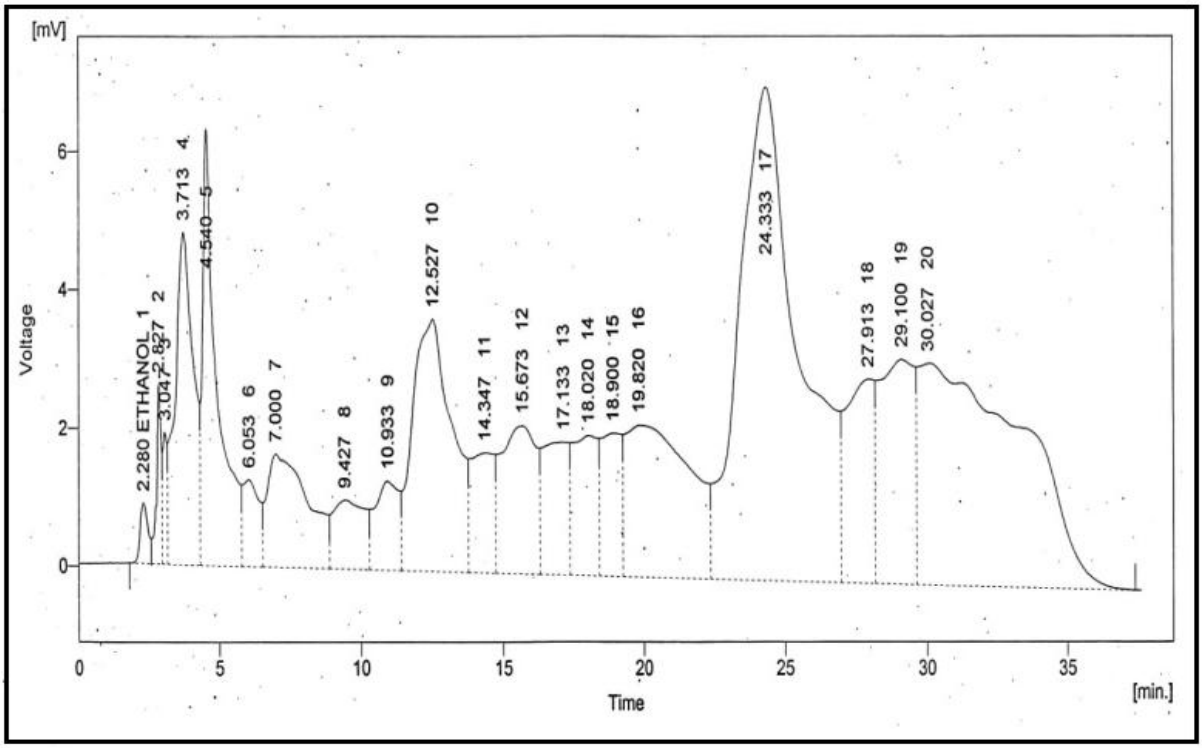

Fig. 1. HPLC Chromatogram of ethanol extract of *Tamarix Aphylla*.

The highest body weight and liver weight was observed in G_3 (39.16±2.19; 5.83±0.5) and G_4 (36.33±1.57; 4.99±0.19) at the dose of 200mg/kg b.wt and 500mg/kg b.wt respectively. The results were significant increase ($p< 0.05$) as compared with the G_2 group. Previous results on seed melon extract on CCl_4 induced hepatic fibrosis in mice showed that the mice in the model group (CCl_4) significantly increase the liver weight and body weight ($p< 0.05$) and decrease in the plant extracted group (Zhan *et al.*, 2016).

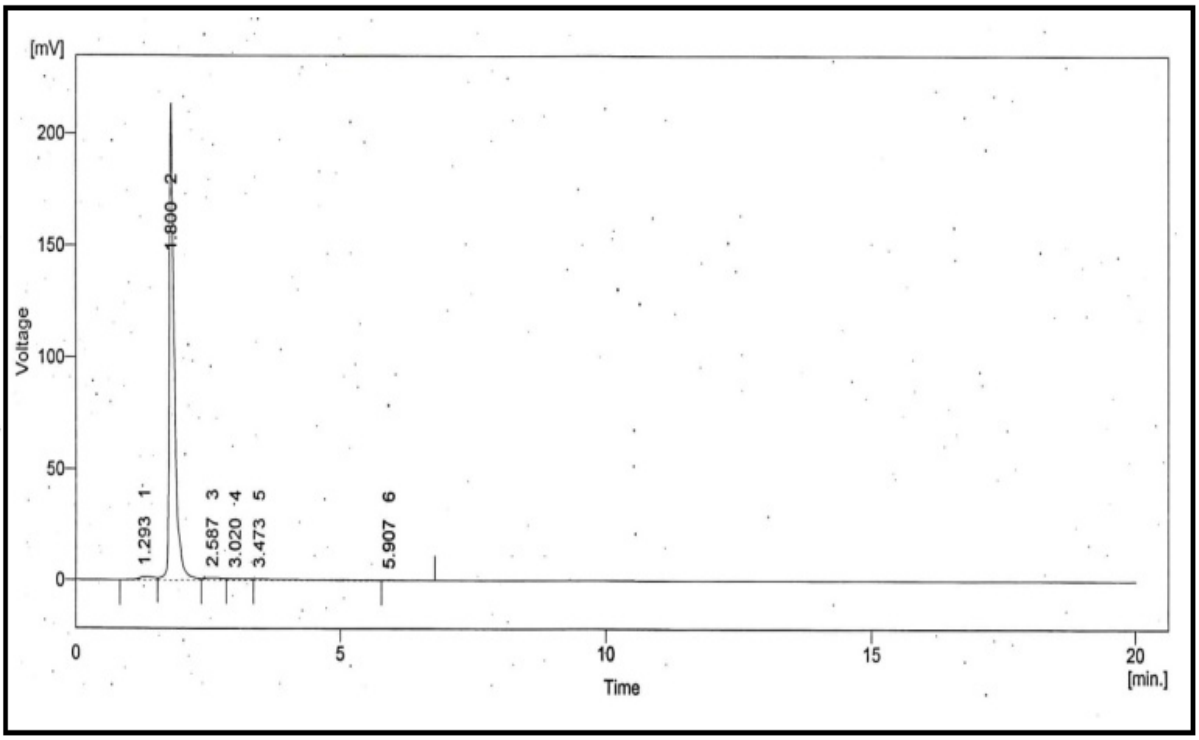

Fig. 2. HPLC chromatogram of kaempferol for *Tamarix Aphylla*.

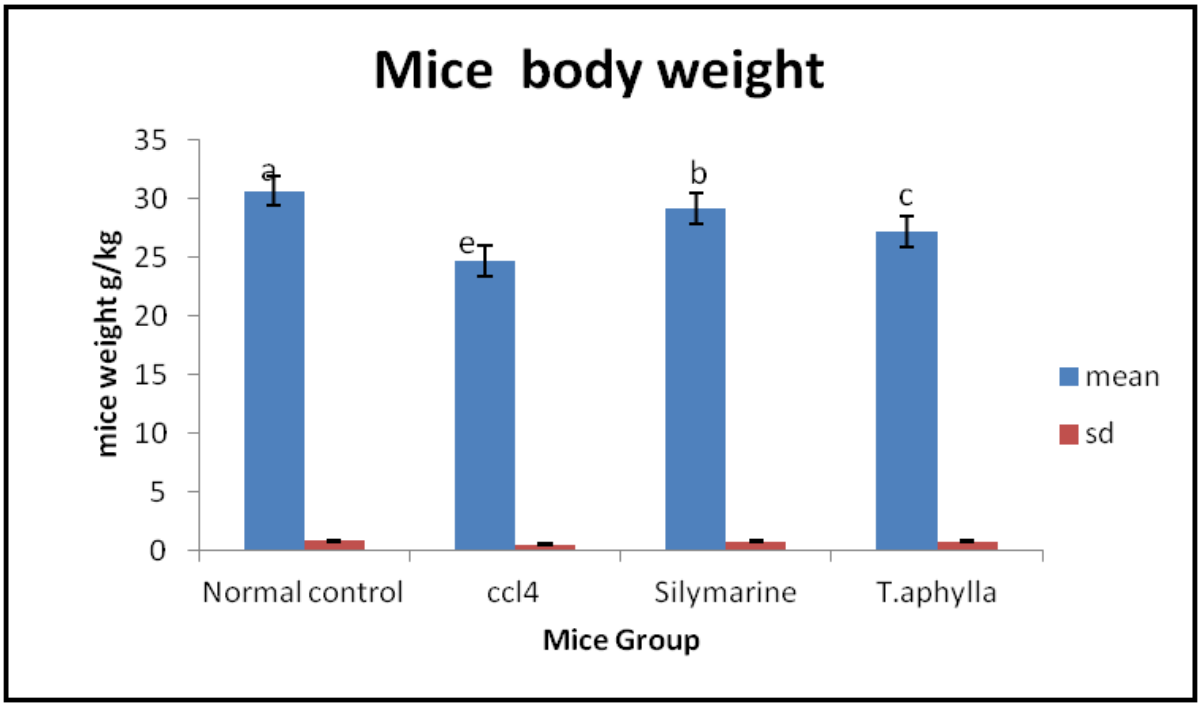

Fig. 3. Effect of ethanol extract of *Tamarix aphylla* on mice body weight.

Biochemical estimation

The effect of *Tamarix aphylla* leave extract (500mg/kg b.wt) on ccl4 injected mice of serum marker enzymes are shown in (Table. 3). The values of AST [(29.667±1.5) IU/L], ALT[(36.333±3.7) IU/L], ALP[(179.5±5.5) IU/L], bilirubin total [(0.68±0.01) IU/L] in control group while the values of AST [(213.6±5.4) IU/L], ALT[(264.16±5.9) IU/L], ALP[(306.3±4.9) IU/L], bilirubin total [(1.41±0.3)IU/L] were significantly increased in CCl_4 treated group ($p< 0.05$) when compare with the control group.

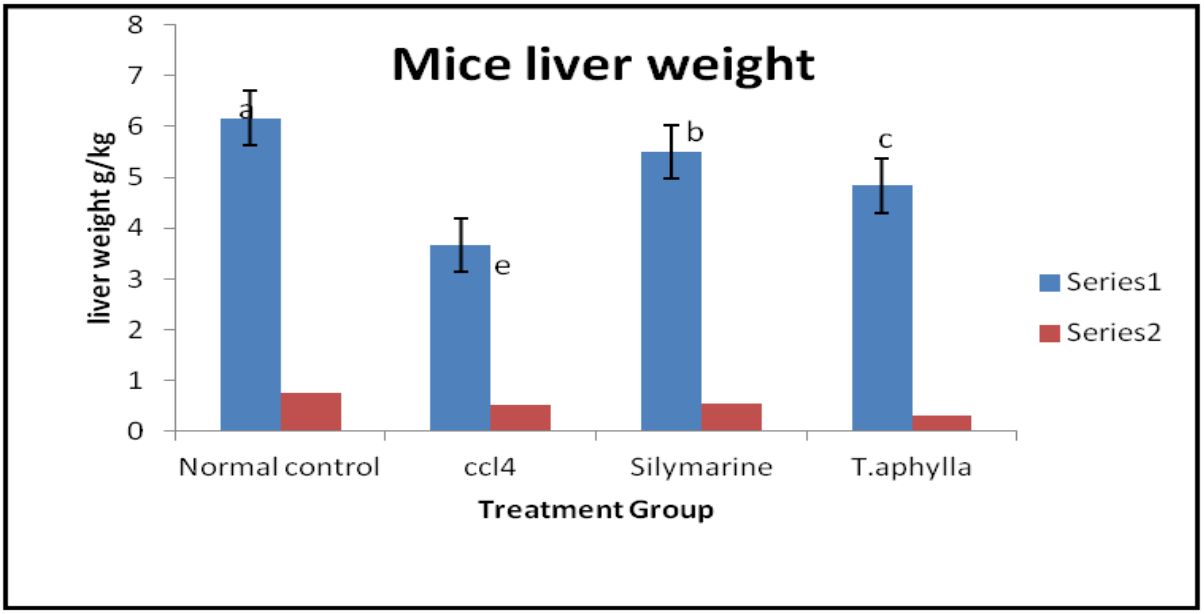

Fig. 4. Effect of ethanol extract of *Tamarix aphylla* on mice liver weight.

The treatment of mice with ethanol extract of *Tamarix aphylla* leave at (500mg/kg b.wt) showed significantly reduction in AST [(43.35±2.65) IU/L], ALT [(53.5± 2.8) IU/L], ALP [(178.33±2.1) IU/L], bilirubin total [(0.75±0.03) IU/L] levels ($p< 0.05$).

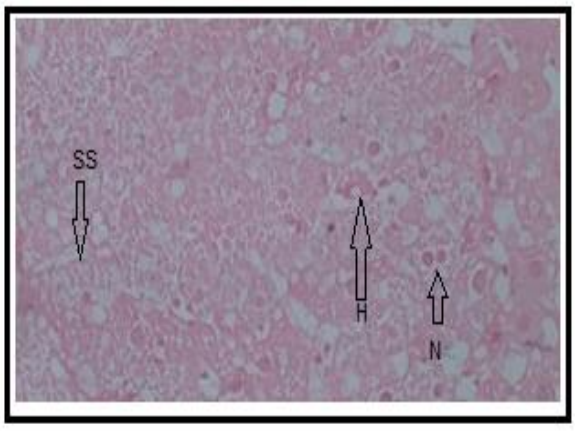

Fig. 5. Histology of normal liver cells (normal control group). Microscopic study of normal liver cell showed, normal nucleus (N), hepatocytes (H) and sinusoidal spaces (SS) but not found any inflammation and nacrosis, blooming or degeneration.

However silymarin treated animals also showed significant ($p < 0.05$) inverted values of liver marker enzymes AST [(35.33±3.82) IU/L], ALT [(43.46±3.26) IU/L], ALP [(163.83±5.88) IU/L], bilirubin total [(0.55±0.04) IU/L] when compare with the CCl_4 treated groups. Yusufoglu and Algasoumi have investigated the potential role of various *Tamarix aphylla* leave extract in the prevention and/or treatment of many alignments (Yusufoglu and Algasoumi 2011) interestingly this is the first time evaluating the hepatoprotective activity of ethanol extract of *Tamarix aphylla* leaves against CCl_4 liver injury in mice.Result were expressed as Mean± SD (n=6) each value is considered statistically significant at ($p < 0.05$). group sharing the same superscripts are not statistically different. AST (aspartate transaminase); ALT (alanine transaminase); ALP (alkaline phosphate); Bil.T (bilurubin total); Bilrubin direct or Bilrubin indirect. G1: conrol; G2: CCl4; G3: Silymarine; G4: Tamarix aphylla.

Measurement of CCl_4 mediated Oxidative stress

The enzymatic antioxidants activity such as catalase (6.91 ± 0.30), SOD (10.26 ±1.07), GPX (1.05 ± 0.08) in CCl_4 treating groups showed significantly reduce ($p < 0.05$) in the liver tissues when contrast with the catalase (12.56±0.92), SOD (25.37± 1.11), GPX (2.96± 0.37) values of control group.

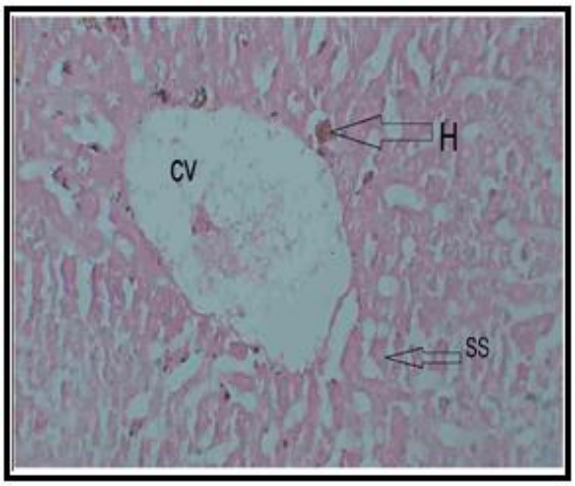

Fig. 6. Hispathological study of disease group CCl_4 treated showed severe inflammation, hemorrhagic necrosis and degeneration of central vein (CV), hepatocytes (H) and sinusoidal spaces (SS) Normal structure of nucleus is not seen and degraded the hepatocyte cell.

Tretment with the *Tamarix aphylla* at the dose of (500mg/kg) resulted in the significant increase in catalase (12.41±0.30), SOD (19.08±1.25), GPX (2.47 ± 0.17) However treatment with the silymarin catalase (11.71±0.32), SOD (21.18± 1.05), GPX (1.90 ±0.05) activity was increase when contrast with the CCl_4 treated mice. Analysis of lipid per oxidation (22.51±1.15) level by CCl_4 induction showed a significant ($p < 0.05$) increase. However, treatment with leave extract of *Tamarix aphylla* (500mg/kg), the values of LPO (13.75 ±0.10) as well as silymarine (200mg/kg) (12.77 ±0.30) significantly ($p < 0.05$) prevented the high level of LPO which was brought to near normal (10.39± 0.05) shown in (Table 4). Malondialdehyde (MDA) content in liver with CCl_4 treated mice was significantly increase (7.49± 0.15) than that of control group (2.49± 0.03) However, MDA level showed significantly decrease ($p < 0.05$) in *Tamarix aphylla* (2.49± 0.03) treated group or standard silymarin (3.05 ±0.06). (Sekkien *et al.*, 2018) reported that the biochemical evaluation of Tamarix nilotica on lipid peroxidation increase level in CCl_4 treated mice and catalase activity was significantly decreased ($p < 0.05$) and lipid peroxidation level also decrease in *Tamarix nilotica*.

Result were expressed as Mean± SD (n=6) each value is considered statistically significant at ($p < 0.05$). Group sharing the same superscripts are not statistically different. G1: conrol; G2: CCl4; G3: Silymarine; G4: Tamarix aphylla; Catalase (U/mg of protein), GPX: Glutathione per oxidase (U/mg of protein), SOD: Supper oxide dismutase (U/mg of protein), MDA- nm/mg of protein; LPO: Lipid per oxidase.

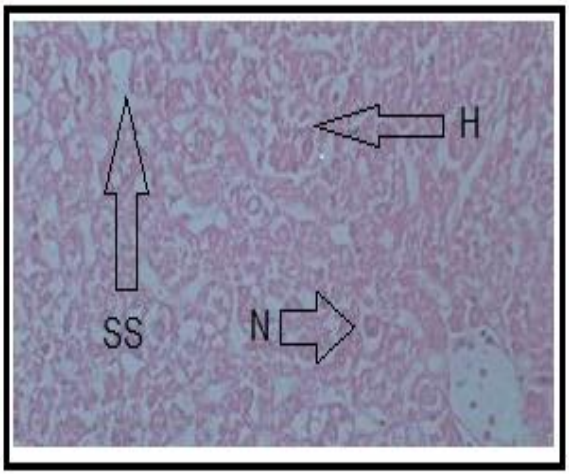

Fig. 7. Histopathology study of standard Silymarin group. The photographs of liver section stained with H&E magnification, x400. Photomicrograph of standard group showed a hepatocytes (H) and sinusoidal spaces (SS) and nucleus (N), with normal cell and not any inflammation.

Light Microscopic Examination

The liver anatomy of the gross section indicated the normal articulation in the control group. Hepatic cell and central veins were also well organized in cord clearly in sinusoid space, while the parenchyma covered the portal triads and liver cell section showed the prominent lining. (Fig.5.) the CCl_4 (1ml/g body weight) treated group (Fig.6.) Showed the tissue necrosis and inflammation and the section were severe liver damage and showing the congestion of

microvesicular and macrovascular steatosis. *Silymarin* treated group (200mg /g b.wt) (Fig. 7.) showed no any inflammation in liver cell and exhibited the defense to liver damage, while the therapy of ethanol extract of *Tamarix aphylla* leave (500mg /g body weight) was showed the minor expansion of sinusoids with no inflammation in parenchyma cell and revealed protection from necrosis (Fig.8.).

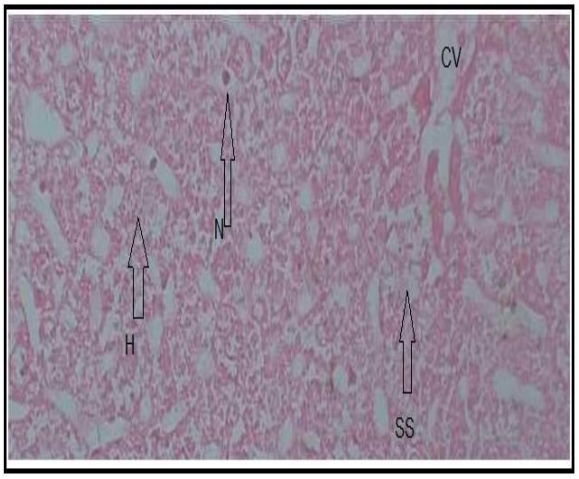

Fig. 8. The photographs of liver section stained with H&E magnification, x400. Histopathology study of ethanol extract of *Tamarix aphylla* group showed a hepatocytes (H) and sinusoidal spaces (SS) and nucleus, with normal cell and not any inflammation.

Thus extract had hepatoprotective activity which is revealed by the histopathological study. Our results compare with the (Kuppan *et al.*, 2011), histopathological results showed that liver of paracetamol intoxicated mice showing wide necrosis across the cell and necrosis, degeneration in hepatic architecture and loss of cellular boundaries while therapy with C. ternatea was effective in decreasing the rate of necrosis against induce paracetamol lesion and normal liver architecture was exhibited.

Conclusion

The findings of the study showed that *Tamarix aphylla* leaves have good phytochemical potential. In vivo studies, *Tamarix aphylla* significantly reduced the level of liver marker enzymes (ALT, AST, ALP, and bilirubin) and decrease the rate of lipid peroxidation and MDA level in mice. Furthermore, histopathological results showed that the highest hepatoprotective activity of *Tamarix aphylla* leaves was seen.

This study is limited by some aspects that the observation was carried out only on male mice, thus, we cannot leave out the possibility that sex influences the effect of *Tamarix aphylla* leaf extract on CCl_4 and survival mechanisms. Furthermore, there is a need to explore more the action of *Tamarix aphylla* examine the mechanism of actions with other therapeutic activities. It is recommended that extract of *Tamarix aphylla* could be used to reduce the rate of liver fibrosis or liver related diseases.

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Ethics approval and consent to participate

All the experiments were performed in strict agreement according to an ethical review committee and guidelines for the purpose of experiments on animals (Ref. No.GCUF/ERC.1962; IRB No. 562).

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